Neospora caninum and Toxoplasma gondii antibodies in red foxes (Vulpes vulpes) in the Czech Republic

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This is the first prevalence study of Neospora caninum and T. gondii antibodies in red foxes in the Czech Republic. The results obtained show that red foxes are exposed at different levels to both protozoan infections, and thus could play an important role in the transmission cycle of N. caninum and T. gondii in sylvatic cycle.

INTRODUCTION

Neosporosis is a serious disease of cattle and dogs worldwide [1]. Definitive hosts of N. caninum are dogs and other canids such as dingo, coyote and grey wolf. T. gondii is a coccidian parasite with cats and other felines as the definitive host, and warm-blooded animals as the intermediate hosts. Experimentally-infected animals remained asymptomatic irrespective of T. gondii strain [2].

Since foxes are at the top of food pyramid, their prevalence reflects the situation in the environment. Although foxes have not been proved as the definitive host of N. caninum based on experimental infection [3, 4], they play important role in maintaining N. caninum and T. gondii infection in the sylvatic cycle. In Europe, there are reports of N. caninum and T. gondii seroprevalence in red foxes (Vulpes vulpes) in the range of 0% – 3.2% and 35% – 100%, respectively. There are no reports from the Czech Republic which is why the aim of this study was to examine the sera of red foxes from the Czech Republic for N. caninum and T. gondii antibodies.

MATERIALS AND METHOD

Between February – March 2012, blood samples were collected from 80 red foxes from 8 regions of the Czech Republic as the part of survey for the detection of post- vaccination anti-rabies antibodies. The blood samples were centrifuged and sera stored at -20°C until assayed. The area of the regions in km² and the number of samples taken in individual regions are summarized in Table 1. During 2012, 75,296 red foxes were hunted in the Czech Republic [5].

The sera were tested for N. caninum and T. gondii antibodies by the reference method of indirect fluorescent antibody test (IFAT) using a commercially available N. caninum and T. gondii substrate slide (VMRD Inc., Pullman, USA) and anti-dog IgG conjugate (Sigma Aldrich, St. Louis, Missouri, USA). The sera were diluted in a double dilution starting at 1:50 as the basic dilution; a titre of 50 was considered positive for both parasites. Neospora caninum positive and negative control canine sera (both VMRD) were used. Sera from dogs simultaneously positive in both latex agglutination test (LAT) and IFAT and IFAT negative sera from puppies of a laboratory Beagle served as T. gondii positive and negative controls, respectively. Both positive and negative control sera were included in each slide. In the case of positive samples, green antigens of T. gondii were observed under an immunofluorescence microscope.

The seroprevalence of antibodies to N. caninum were also investigated by competitive enzyme linked immunosorbent assay (cELISA, VMRD Inc.), according to the manufacturer’s
Antibodies to *N. caninum* were detected by IFAT in 3 (3.8%) of 80 foxes with titre 50. Two of these positive samples were collected from a district in South Bohemia and one from the Ústí nad Labem district. These positive samples were simultaneously positive for *T. gondii* antibodies by both methods (in IFAT with titres 3,200, 400 and 400). In cELISA, *N. caninum* antibodies were detected in 2 (2.5%) of 80 foxes with inhibition 42.7% and 30.2%. These samples were collected in the districts of South Bohemia and Ústí nad Labem and were simultaneously positive for *T. gondii* antibodies by both methods (in IFAT with titres 800 and 200, respectively).

Antibodies to *T. gondii* were detected by IFAT in all 80 foxes with titres in the range 50 – 6400 (Tab. 1). In ELISA, *T. gondii* antibodies were detected also in all 80 foxes with S/P ranging from 34% – 133%. This is the first prevalence study of *N. caninum* and *T. gondii* antibodies in foxes in the Czech Republic.

**RESULTS**

DISCUSSION

The results of this study indicate that *T. gondii* is fairly common (100%) in foxes in the Czech Republic in contrast with *N. caninum* that was detected only in 3.8% and 2.5% of foxes by IFAT and cELISA, respectively. Five samples had antibodies against both parasites and *Neospora caninum* titres were less than to the *Toxoplasma gondii* antibody titres. A cross-reactivity of coccidia might have had an impact on the results, but co-infection with both parasites cannot be ruled out either. It would be interesting to discover the risk factors, such as gender and age of animals, unfortunately, these data are not available.

In Spain, antibodies to *N. caninum* were demonstrated by cELISA and confirmed by IFAT in 3.2% of 95 red foxes [6], similar to the presented study; also similar to this study, a high *T. gondii* seroprevalence (100%) was found in 123 foxes in Belgium [7]. Antibodies to *T. gondii* were detected by immunoblot analysis in 74.5% of 204 and 84.7% of 176 red foxes in Germany [8], and by IFAT in 53.4% of 191 foxes in Italy [9]. Higher *T. gondii* prevalence compared to *N. caninum* was detected also in other countries. In Hungary, antibodies to *T. gondii* and *N. caninum* were found by direct agglutination test (DAT) in 68%, and by iscom-ELISA in 1.5% of 337 red foxes, respectively [10]. In Sweden, antibodies to *T. gondii* were found by DAT in 38% of 221 red foxes, but none of the foxes had antibodies to *N. caninum* by using iscom-ELISA [11]. In Austria, 35% of 84 red foxes tested by IFAT were positive for *T. gondii* antibodies, but negative for *N. caninum* antibodies [12]. The results of seroprevalence studies may differ according to the method, number of tested animals, location and year of sampling.

*T. gondii* antibodies were found in foxes in all districts of the Czech Republic, while *N. caninum* antibodies only in foxes from 2 districts (South Bohemia and Ústí nad Labem). It would be interesting to study possible risk factors, since there are differences in the size of the districts (Tab. 1), natural conditions and presence of potential sources of these parasites. In the Czech Republic, Hurkova and Modry [13] detected *T. gondii* and *N. caninum* by PCR directly in the tissues of 1.3% and 4.6% of 152 red foxes, respectively.

Foxes can be infected by drinking water contaminated with *T. gondii* or *N. caninum* oocysts or by hunting the animals (especially small mammals such as rodents and hares) that are infected with parasites. Kidawa and Kowalczyk [14] examined the stomachs of 224 foxes in Poland. They found that the most frequent prey were voles (46.9% of the diet by volume) and brown hares (10.7% of diet by volume). In the Czech Republic, *T. gondii* and *N. caninum* antibodies were detected in 21% and 39% of 333 hares, respectively [15]; these animals could therefore serve as the source of infection in foxes.

Stray dogs and cats shed *N. caninum* and *T. gondii* oocysts, respectively, and contaminate the environment for a long time. Prey animals could be continuously infected and serve as the main source of infection for carnivorous. Therefore, it is important to continue monitoring *N. caninum* and *T. gondii* infection in foxes, small mammals and hares to assess the actual situation.

Acknowledgements

The authors express their thanks to Lenka Žáčková for assistance with the sample examinations.
Table 1. Distribution of antibody titres to *Toxoplasma gondii* in red foxes in 8 regions of the Czech Republic

<table>
<thead>
<tr>
<th>District</th>
<th>Area (km²)</th>
<th>Number of animals</th>
<th>Total positive</th>
<th>T. gondii – IFAT (titres)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jihočeský</td>
<td>10 056</td>
<td>15 15</td>
<td>1 3 5 5</td>
<td>1 1</td>
</tr>
<tr>
<td>Karlovars ký</td>
<td>3 314</td>
<td>6 6</td>
<td>1 1</td>
<td>1 1</td>
</tr>
<tr>
<td>Královohradecký</td>
<td>4 758</td>
<td>9 9</td>
<td>2 1 5</td>
<td>1 1</td>
</tr>
<tr>
<td>Liberecký</td>
<td>3 163</td>
<td>21 21</td>
<td>3 3 3</td>
<td>2 1</td>
</tr>
<tr>
<td>Pardubický</td>
<td>4 519</td>
<td>4 4</td>
<td>1 1</td>
<td>1 1</td>
</tr>
<tr>
<td>Plzeňský</td>
<td>7 561</td>
<td>1 1</td>
<td>1 1</td>
<td>1 1</td>
</tr>
<tr>
<td>Středočeský</td>
<td>11 016</td>
<td>16 16</td>
<td>4 3 4</td>
<td>1 1</td>
</tr>
<tr>
<td>Ústecký</td>
<td>5 335</td>
<td>8 8</td>
<td>3 2</td>
<td>1 1</td>
</tr>
<tr>
<td>Total (%)</td>
<td>49 722</td>
<td>80 80</td>
<td>11 (14%)</td>
<td>16 (20%)</td>
</tr>
</tbody>
</table>

IFAT – indirect fluorescent antibody test.

REFERENCES